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The mechanism of UVB irradiation induced-apoptosis in cataract

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Abstract Cataract is the most common eye disease that causes blindness in patients. Ultraviolet B (UVB) irradiation is considered an important factor leading to cataract by inducing apoptosis in human lens epithelial cells (HLECs), but the mechanism is currently unclear. In this study, we investigated HLECs under different intensities of UVB irradiation and different exposure time. The annexin V-FITC/propidium iodide staining results showed that UVB irradiation could efficiently lead to HLECs apoptosis in time- and dose-dependent manner. The expression of pro-apoptotic Bax gene was promoted by UVB irradiation, while anti-apoptotic Bcl-2 gene expression was inhibited at both transcript and protein levels. Notably, the ratio of Bax/Bcl-2 displayed a high and positive correlation to the proportion of apoptotic HLECs. Mitochondrial dysfunction was also observed with rapid loss of potential ($\Delta\Psi_m$), as well as changes of the levels of reactive oxygen species, malondialdehyde, total antioxidative capabilities, and superoxide dismutase. In caspase pathway, the level of caspase-3 protein increased after UVB irradiation. All these discovered changes may play important roles in UVB-induced HLECs apoptosis, and would be helpful in understanding the mechanism of UVB-induced cataract and providing potential prevention and treatment strategies.

Keywords UVB irradiation · Cataract · Apoptosis · Caspase-3 · Human lens epithelial cells (HLECs)

Introduction

Cataract is one of the most prevalent eye diseases and a major cause of legal blindness in the world [1]. Studies have shown that photo-oxidation by ultraviolet (UV) was connected to cataract development, and ultraviolet B (UVB) (295–320 nm wavelength) irradiation was one of the most important factors of cataractogenesis [2–4]. Eye exposure to UVB irradiation may induce unscheduled DNA syntlhesis, DNA damage and repair, decrease of glutathione, enhancement of prostaglandin synthesis, impairment of membrane pumps, and inactivation of a number of metabolic enzymes [5]. Wölfle et al. investigated the UV-protective and antioxidant properties of luteolin in human keratinocytes and revealed that UVB radiation could cause endogenous damages by generating reactive oxygen species (ROS) that would damage lens DNA and proteins, resulting in loss of transparency [6]. It is believed that UVB-induced cataract formation initiates with damages to human lens epithelial cells (HLECs), and apoptosis in HLECs is an early event in the development of UVB-induced cataract.

It has been well characterized that apoptosis is mainly regulated by Bcl-2 family proteins, among which Bcl-2 protein is the most studied member that has negative control effect in cellular apoptotic pathway [7–11]. A Bcl-2-homologous protein, Bax, can reverse the suppressing effect of Bcl-2 in apoptosis. Gross et al. discovered that there was a major checkpoint in the common portion of mammalian cell death pathway, which depends on the ratio of pro-apoptotic Bax to anti-apoptotic Bcl-2 levels [12].

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The mechanism of apoptosis is evolutionarily conserved and related to mitochondrial dysfunction and caspase pathway [12].

Ultraviolet B irradiation-induced apoptosis is regulated by a number of molecular processes, which target mitochondria and activate mitochondria-initiated cell death pathway. These processes include change of $\Delta\Psi_m$, production of ROS, opening of the permeability transition pore (PTP), and release of cytochrome c (Cyt c). Ricci et al. showed that during apoptosis, mitochondria lost transmembrane potential rapidly and generated ROS [13]. Wu et al. discovered that $\Delta\Psi_m$ was decreased after exposing of HLE B-3 cells to different intensities of UVB. $\Delta\Psi_m$ decreased with increased UVB irradiation, while the level of ROS was increased [1]. Previous studies also showed that oxidative stress is a major cause in the initiation and progression of cataracts. It is believed that lenses have evolved antioxidant systems to counter the toxic damage of ROS or free radicals, including both antioxidants and antioxidant enzymes such as reduced glutathione (GSH), total antioxidative capability (T-AOC), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione S-transferase (GST), catalase (CAT), and glutathione reductase/peroxidase (GR/Gpx) [14–16]. Zhang et al. measured the levels of MDA and T-AOC, as well as the activities of antioxidant enzymes GSH, NAC, CAT, and SOD to determine oxidative damages of Paraquat to HL-60 cells [17]. The changes of MDA and T-AOC were also measured due to treatment of ursodeoxycholic acid, which finally determined to be contributed to prevent selenite-induced oxidative stress and alleviate cataract formation [18]. Prabakaran et al. also evaluated the protective effect of hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rat by assessing the activities of enzymic antioxidant enzymes SOD, CAT, GPx, GST [19].

Besides mitochondrial dysfunction during cell apoptosis, caspase activities may be activated by the released Cyt c from mitochondria, which can in turn affect the function of mitochondria [12]. As a matter of fact, some in vitro studies have demonstrated the activation of caspase-3 during apoptosis along with oxidative damage by the production of ROS [20, 21]. It is believed that caspase-3 plays a pivotal role in the execution of apoptosis [22, 23]. The role of caspase-3-mediated apoptotic pathway in the development of cataract was also investigated [24–26]. For example, Anderson et al. found the caspase-3 activity decreased in human lens epithelium in posterior subcapsular cataract [24]. Yao et al. investigated the effect of the herbal ingredient parthenolide against oxidative stress-induced apoptosis in HLECs and the results indicated the activation of caspase-3 and caspase-9 was inhibited, suggesting a potential protective effect of parthenolide against cataract formation [26].

To date, there have been several studies on UVB irradiation-induced apoptosis in cataract, but few have focused on finding the underlying mechanisms such as the regulation of apoptosis-related genes and the two major apoptosis executing downstream phenotypes: mitochondrial dysfunction and caspase activities [1, 27, 28]. Thus, our study was aimed to investigate the above-mentioned details of the apoptotic process in HLECs after UVB irradiation.

Materials and methods

Cell culture

A human lens epithelial cell line (HLEC) was used in this study. The cells were cultured in RPMI-1640 medium (Hyclone, China) supplemented with 10 % fetal bovine serum, 1 % glutamine, and 1 % penicillin/streptomycin, seeded when they grow into the log phase, and grown to 80 % confluence for experiments.

UVB exposure

The UV exposure was executed by a UV-B radiometer (Photoelectric Instrument Factory, Beijing Normal University, China) with a total output of 2 W/m^2 (297 nm). Before UVB irradiation, the HLECs were washed twice to remove residual serum and non-attached cells using phosphate buffered saline (PBS, pH 7.4). There were 14 different combinations of exposure time and UVB intensity of HLEC treatment: 1 W/m^2 for 5, 15, 30, 45, 60, 90, and 120 min, respectively; 2 W/m^2 for 5, 15, 30, 45, 60, 90, and 120. After UVB irradiation, the HLECs were re-suspended with new medium and cultured in an incubator (Thermo, USA) at 37 °C with 5 % CO_2.

Annexin V-FITC/propidium iodide (PI) staining

The HLECs were stained with Annexin V-FITC and PI (Annexin V-FITC Apoptosis Detection Kit, BD Biosciences, USA), and evaluated by flow cytometry (BD Biosciences, USA) to assess the development of apoptosis. Briefly, each UVB irradiated HLECs was collected and re-suspended in 190 μL of 1 X Annexin-binding buffer, then 5 μL of Annexin V-FITC and 10 μL of PI solution were added and incubated according to the manufacturer's instruction. All samples (each sample 1×10^4 cells) were analyzed by flow cytometry, and data collected in each window were designated FL1-channel and FL2-channel, respectively. To set the quadrants and adjust the compensation between the flow cytometer and the detectors, controls including unlabeled cells, cells labeled with Annexin only, and cells labeled with PI only were used.

Determination of mitochondrial membrane potential ($\Delta\Psi_m$)

The $\Delta\Psi_m$ was measured by flow cytometry using cationic dye JC-1 assay kit. JC-1 can be used to measure $\Delta\Psi_m$ at high sensitivity because it exists as a green-fluorescent monomer at low membrane potential, and forms red-fluorescent aggregates JC-1 at higher membrane potential. Briefly, HLECs were incubated in 1 mL medium containing 0.5 mL JC-1 staining working solution (Beyotime, China) at 37 °C for 30 min. The cells were then washed twice with JC-1 staining buffer and analyzed using a flow cytometer (BD Biosciences, USA) at 488 nm excitation wavelength. Data were collected at an emission wavelength of 530 and 590 nm for green fluorescence (FL-1 channel) and for red fluorescence (FL-2 channel), respectively. The baseline fluorescence was evaluated by non-staining control cells.

ROS assay

ROS levels were measured using the ROS assay kit (Cat. No. S0033, Beyotime Institute of Biotechnology, Jiangsu, China) as previously reported [20]. In brief, the HLECs were collected and incubated with medium containing 10 mM DCFH-DA at 37 °C for 30 min. After incubation, the cells were washed with PBS for three times. Cellular fluorescence of ROS production was quantified by fluorescence microplate reader at 488 nm excitation and 525 nm emission wavelengths. The plate was kept away from light to minimize fading of the fluoroprobe.

Determination of MDA, T-AOC, and SOD activities

To determine the antioxidant levels, we used a T-AOC Assay Kit utilizing ferric reducing ability of plasma (FRAP) method (S0116, Beyotime, China). The procedure was the same as previously reported [29]. MDA, a product of lipid peroxidation, was also analyzed by an assay kit (S0131, Beyotime, China) following the manufacturer's protocol. MDA levels were measure at 532 nm for the formation of stable chromophoric with thiobarbituric acid (TBA).

The activity of SOD was determined using Total SOD Assay Kit with WST-1 (S0102, Beyotime, China), which was based on the ability of SOD to inhibit the oxidation of oxymine by O_2^- produced from the xanthine–xanthineoxidase system. We defined one unit of SOD activity as reduced reduction of the absorbance by 50 % at 550 nm wavelength.

Real-time PCR analysis of Bcl-2 and Bax expression

After each UVB treatment, Bcl-2 and Bax mRNA abundances in HLECs were investigated by real-time quantitative PCR. Total RNA was isolated with Trizol reagent (Invitrogen) according to the manufacturer's protocols. The cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo). Real-time quantitative PCR was performed in 25 μL reaction mixtures with 12.5 μL SYBR Green Mix, 0.5 μL forward & reverse primers, 9.5 μL RNase-free water, and 2 μL cDNA. The PCR primers for amplification of Bcl-2 were: 5′-AGCT GAGCGAGTGTCTCAAG-3′ (forward) and 5′-TGTCC AGCCCATGATGGTTC-3′ (reverse). The PCR primers for amplification of Bax were: 5′-AGACCGAAGTCCG CAGAACC-3′ (forward) and 5′-GAGACCACACTGCC CTGTTG -3′ (reverse). GAPDH was used as internal control and amplified using forward primer 5′-CACCC ACTCCTCCACCTTTG-3′ and reverse primer 5′-CCAC CACCCTGTTGCTGTAG-3′. Real-time PCR reactions were performed on an ABI-7300 (ABI, USA) using the following program: DNA denaturing at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and then 60 °C for 45 s. The expressions of Bax and Bcl-2 were determined by the $2^{-\Delta Ct}$ analysis method comparing to GAPDH gene.

Western blot assay

Total proteins of HLECs were extracted using RIPA lysis buffer (50 mM Tris–HCl pH 7.5; 1 mM EDTA; 150 mM NaCl; 0.5 % sodium deoxycholate; 1 % Triton-X-100; 0.1 % SDS). Extracted proteins were quantified with Bio-Rad Protein Assay Kit. 20 μg of proteins from each sample were analyzed by SDS–polyacrylamide gel electrophoresis (PAGE). Immunoblotting was done including the following antibodies: Bax (Santa, Sc-493), Bcl-2 (Santa, Sc-492), GAPDH (Cell Signaling, 5174), and Caspase-3 (Abcam, ab32351).

Statistical analysis

Each sample was analyzed with three replicates and the average values were calculated. Statistical analyses including independent *t*-test and correlation analysis (Pearson's r) were conducted using SPSS 17.0 software. Data were presented as mean ± SD, and $p \leq 0.05$ or $p \leq 0.01$ were considered significant.

Results

Apoptosis assessment

To determine the effects of UVB irradiation on HLECs, the percentage of apoptotic cells in each sample was determined (Table 1). At 1 W/m^2 of UVB irradiation, apoptosis

Table 1 The percentage of apoptosis cells in each group by flow cytometry analysis

Comparisons	Means (%)	*p* value*
Control group	0	–
First experiment		
1 W/m^2 5 min UVB irradiation	0	–
1 W/m^2 15 min UVB irradiation	0	–
1 W/m^2 30 min UVB irradiation	3.73 ± 0.04	3.81E−08
1 W/m^2 45 min UVB irradiation	6.13 ± 0.24	5.00E−06
1 W/m^2 60 min UVB irradiation	10.47 ± 0.49	9.41E−06
1 W/m^2 90 min UVB irradiation	18.23 ± 0.24	9.17E−08
1 W/m^2 120 min UVB irradiation	25.83 ± 0.69	1.50E−06
Second experiment		
2 W/m^2 5 min UVB irradiation	0	–
2 W/m^2 15 min UVB irradiation	2.07 ± 0.18	1.42E−04
2 W/m^2 30 min UVB irradiation	4.43 ± 0.09	3.06E−07
2 W/m^2 45 min UVB irradiation	8.57 ± 0.24	1.88E−06
2 W/m^2 60 min UVB irradiation	14.13 ± 0.36	1.07E−06
2 W/m^2 90 min UVB irradiation	19.67 ± 0.22	3.09E−08
2 W/m^2 120 min UVB irradiation	30.70 ± 0.53	1.80E−07

* *p* value represents the comparison between the experiment group and control group

started in HLECs after 30 min of UVB irradiation, and the percentage of apoptotic cells increased significantly following extended UVB irradiation. At 2 W/m^2 of UVB irradiation, about 2.07 % HLECs became apoptotic after 15 min, and the number of apoptotic cells also increased with more UVB exposure time. At the same exposure time, much more apoptotic cells were found in HLECs under treatment of 2 W/m^2 UVB than those under 1 W/m^2. These results indicated that UVB irradiation could efficiently lead to apoptosis in HLECs in both time- and dose-dependent manner.

Gene expression of Bcl-2 and BAX in UVB irradiated HLECs

Effects of UVB irradiation on mRNA levels of Bcl-2 and Bax in HLECs were investigated by real-time PCR (Fig. 1a). The expression of Bcl-2 gene started decreasing after UVB irradiation, but only became significant after 15 min of exposure under both 1 and 2 W/m^2 irradiation. After 120 min of UVB irradiation, Bcl-2 gene expression decreased to about 0.56-fold compared to that in the control group. Obviously both longer UVB exposure time and stronger UVB intensity could lead to the decrease of Bcl-2 gene expression, although UVB intensity difference was not as significant as different time period since 2 W/m^2 UVB irradiation only had slight more effects on the decrease of Bcl-2 expression than 1 W/m^2 UVB irradiation. Contrary to the decreased Bcl-2 expression, the expression of Bax gene significantly increased after UVB irradiation, became 1.74 ± 0.08 and 2.01 ± 0.05 after 5 min of 1 and 2 W/m^2 UVB irradiation, respectively (Fig. 1b). After 120 min of UVB irradiation, the expression of Bax gene increased to about 14-fold compared to the control group. Similar to Bcl-2 expression, both exposure time and intensity of UVB irradiation played positive roles in the increase of Bax gene expression. These results demonstrated that UVB irradiation could inhibit expression of Bcl-2 gene while significantly increasing the expression of Bax gene. The protein levels of Bcl-2 and Bax showed proportional decrease or increase with their gene expressions when checked by western blot (Fig. 2).

Since Bax/Bcl-2 ratio has been reported to be a critical checkpoint in cell apoptosis, the correlation between the proportion of apoptosis and Bax/Bcl-2 ratio was investigated (Fig. 1c). It was discovered that Bax/Bcl-2 ratio was positively correlated to the proportion of apoptosis with a high correlation coefficient (R^2 was about 0.95). The ratio of Bax/Bcl-2 had much more impacts on the proportion of apoptosis in HLECs treated under 2 W/m^2 UVB than in HLECs treated under 1 W/m^2 UVB.

Determination of mitochondrial membrane potential

To further investigate the effects of UVB on HLECs, mitochondrial membrane potential ($\Delta\Psi_m$) was measured by flow cytometry using the cationic dye JC-1 assay kit (Fig. 3). $\Delta\Psi_m$ of HLECs significantly decreased after 15, 30, 45, 90, and 120 min exposure to 1 W/m^2 of UVB irradiation; while under 2 W/m^2 of UVB irradiation, $\Delta\Psi_m$ started to be significantly decreased after 5 min, indicating that higher UVB intensities were more effective in decreasing $\Delta\Psi_m$ than lower UVB intensities.

ROS assay

Oxidative stress is an initiating factor that plays a vital role in the development of cataract. To evaluate the oxidative stress in HLECs after exposure to UVB irradiation, a ROS assay kit was used (Fig. 4a). The measured fluorescence intensity in the control group without UVB irradiation was 111.11 ± 0.053. After treatment with 1 W/m^2 UVB irradiation for different time periods, the fluorescence intensities were 160.70 ± 0.049, 290.83 ± 0.059, 414.18 ± 0.019, 426.89 ± 0.044, 637.97 ± 0.029, 753.35 ± 0.015, and 759.53 ± 0.021 after 5, 15, 30, 45, 60, 90, and 120 min UVB irradiation, respectively. This indicated that the production of ROS increased with longer UVB exposure time. Similar increase of ROS levels could be observed in HLECs under 2 W/m^2 UVB irradiation. Different intensities of UVB irradiation did not seem to affect the ROS level differently. For example, after 15 min of

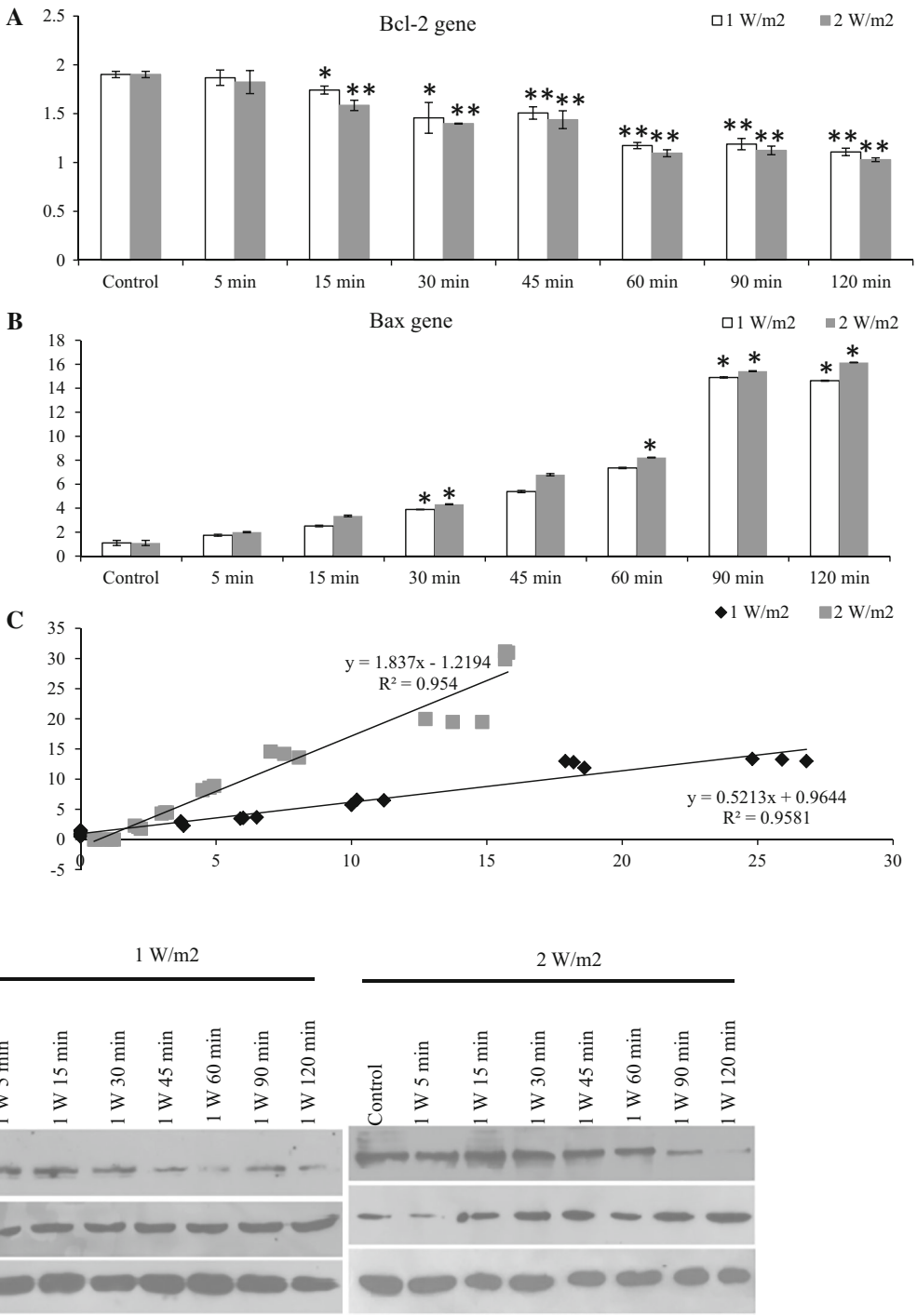

Fig. 1 Real-time quantitative PCR analysis of Bax and Bcl-2 gene in UVB-irradiated HLECs. **a** Expression levels of Bcl-2 gene after UVB irradiation. **b** Expression levels of Bax gene after UVB irradiation. **c** Correlation analysis between the proportion of apoptosis and the ratio of Bax/Bcl-2 in UVB-irradiated HLECs

Fig. 2 Western blot analysis of Bcl-2 and Bax proteins after UVB irradiation in HLECs. GAPDH protein was used as an internal control

2 W/m^2 UVB irradiation, the production of ROS was 161.32 ± 0.042, while the level was 160.70 ± 0.049 after 15 min under 1 W/m^2 UVB irradiation. After 120 min UVB irradiation, the ROS levels in HLECs under 1 W/m^2 UVB and 2 W/m^2 UVB were also very similar. These results clearly revealed that ROS production in HLECs increased with extended exposure time to UVB irradiation.

Measurement of total antioxidative capability (T-AOC) and malondialdehyde (MDA) levels

To determine potential changes of T-AOC and MDA in HLECs upon treatment of UVB, T-AOC and MDA assay kits were used to measure the oxidative stress-initiated physiochemical variations in HLECs. Compared to the

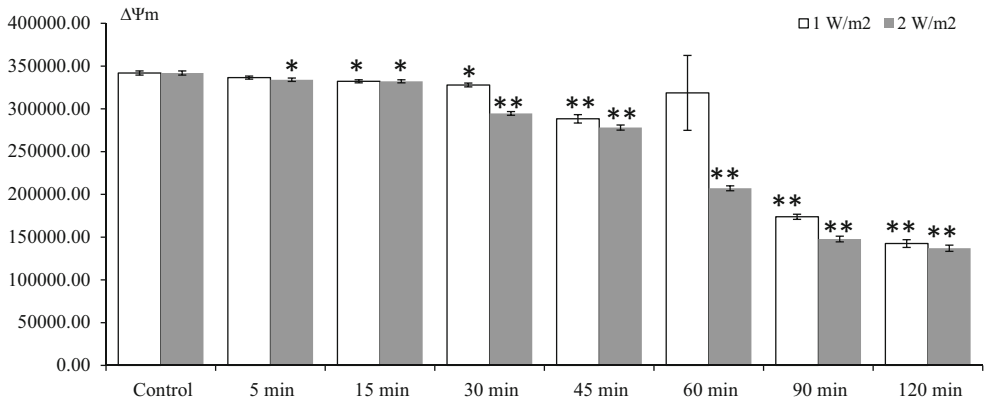

Fig. 3 Mitochondrial membrane potential ($\Delta\Psi_m$) levels in HLECs after treatment with different intensities of UVB irradiation monitored by flow cytometry. *represents p value between the experiment and control group less than 0.05; **represents p value between the experiment and control group less than 0.01

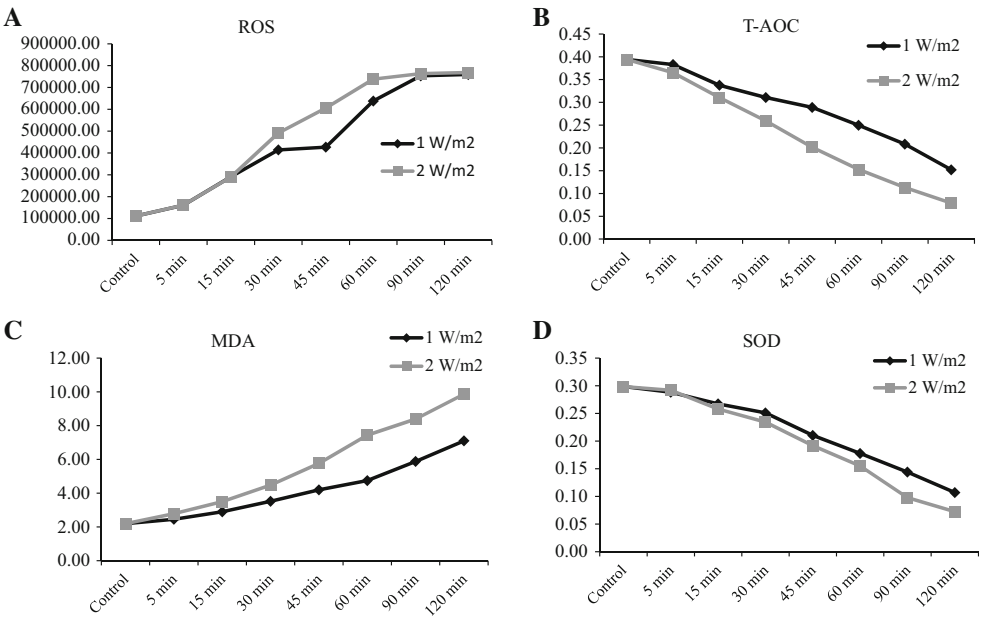

Fig. 4 Evaluation of oxidative stress in mitochondria after treatment with different intensities of UVB irradiation. **a** ROS production in UVB-irradiated HLECs. **b** Levels of T-AOC in UVB-irradiated HLECs. **c** Levels of MDA in UVB-irradiated HLECs. **d** The activity of SOD in UVB-irradiated HLECs

control HLECs, The T-AOC level in HLECs treated with UVB was significantly decreased after 5 min of exposure ($p < 0.05$) under both 1 and 2 W/m^2 irradiation (Fig. 4b). T-AOC levels in HLECs treated with 2 W/m^2 of UVB irradiation were lower than those with 1 W/m^2 of UVB irradiation.

Contrary to the decreased T-AOC level, the MDA levels in HLECs were increased after UVB irradiation. As shown in Fig. 4c, the MDA levels in HLECs increased significantly to 2.45, 2.90, 3.52, 4.20, 4.75, 5.88, and 7.10 nmol/mgprot after treatment of 5, 15, 30, 45, 60, 90, and 120 min of 1 W/m^2 of UVB irradiation, respectively. The MDA levels in HLECs treated with 2 W/m^2 of UVB irradiation were also increased, and were higher than that in 1 W/m^2 irradiation after the same exposure time. Both the reduction in the level of T-AOC and the increase in MDA level in HLECs indicated oxidative damages in mitochondria [27, 28]. These results revealed that UVB irradiation could induce oxidative stress in HLECs in time- and dose-dependent manners.

Measurement of superoxide dismutase (SOD) activity

It was reported that SOD, an antioxidant enzyme, plays an important role in controlling oxidative stress. Thus, the SOD activity in HLECS irradiated with UVB was also measured in the study (Fig. 4d). It was obvious that SOD activity was significantly decreased after UVB treatment, and stronger UVB intensity had more dramatic effects on the decrease of SOD activity. These results further revealed of the induced oxidative stress in UVB irradiated HLECs. Together with changes of ROS, T-AOC, and MDA levels,

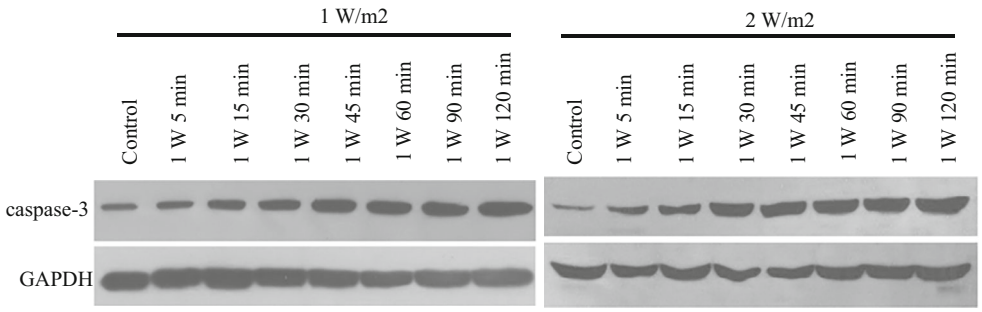

Fig. 5 Western blot analysis of caspase-3 protein after UVB irradiation in HLECs. GAPDH protein was used as an internal control

the UVB-induced oxidative stress likely led to further mitochondrial dysfunction.

Western blot analysis of caspase-3

Caspases are crucial mediators of apoptosis, and among them, caspase-3 is an important apoptosis indicator [30]. In this study, western blot analysis using caspase-3 antibody was performed to investigate the change of caspase-3 in HLECs after UVB irradiation. Western Blot results showed that caspase-3 increased after UVB exposure, and the more exposure time, the more increase of caspase-3 (Fig. 5). After 120 min, the expression level of caspase-3 increased at least 2-fold and 5-fold under 1 and 2 W/m^2 of UVB irradiation, respectively.

Discussion

Cataract is one of the leading causes of irreversible blindness in the world, especially in elder and diabetic populations. The only cure for cataract is surgical removal of the opaque lens and replacement by a clear one. However, the operation is not universally available to all patients, and artificial lenses do not always have the overall optical qualities of healthy lenses [31]. To prevent or delay the onset of cataract and reduce the occurrence of sightlessness, it is very important to find out the mechanism of cataract development. In this study, we investigated the effects of UVB irradiation on HELCs using different exposure time and UVB intensities. The expression of apoptosis regulator genes such as Bcl-2 and Bax was determined; mitochondrial $\Delta\Psi_m$, ROS level, T-AOC, MDA, and SOD activities were measured; the change of the expression of caspase-3 after UVB irradiation was also investigated. These results were useful in explaining the development of cataract that involved regulation of apoptosis-related genes, and the mitochondrial dysfunction and caspase program (Fig. 6).

After different exposure time and intensities of UVB irradiation, HLECs apoptosis was an early event in the development of UVB-induced cataract. UVB irradiation could efficiently lead to HLECs apoptosis in both time- and dose-dependent manner. Many genes are involved in apoptosis, and Bcl-2 gene family is the most important contributor [32, 33]. Among this family, Bcl-2 is a suppressor to apoptosis and Bax is a contributor to apoptosis. We measured the mRNA expression of these two apoptosis-related genes, and the RT-PCR results showed that UVB irradiation inhibited gene expression of Bcl-2 but markedly promoted the expression of BAX gene. Western blot analysis revealed that Bcl-2 protein level was also decreased, while Bax protein level was increased. Since Bax protein counteracts the anti-apoptotic effect of Bcl-2 by forming a heterodimer, the ratio of Bax to Bcl-2 is important in determining the fate of cells after stimulation of apoptotic signals. In this study, Bax/Bcl-2 ratio was highly positively correlated to the proportion of apoptotic cells in HLECs after UVB irradiation (Fig. 1c), indicating that UVB irradiation indeed induced HLEC apoptosis through increased Bax/Bcl-2 ratio.

Apoptosis would lead to damage of mitochondrial functions [12]. Other than generating ATP as energy source, the major functions of mitochondria in lens cells are to maintain lens clarity by keeping the intracellular oxygen content low and preventing proteins and lipids from being oxidized [1]. During apoptosis, mitochondria in lens cells are under increased oxidative stress, which is considered an initiating factor for the onset of cataract development to maturity [34]. Under UVB irradiation, the HLEC mitochondria rapidly lost transmembrane potential ($\Delta\Psi_m$) in both time- and dose-dependent manner, and generated ROS. While ROS are normally produced as by-products during mitochondrial electron transport, heightened levels of ROS would put cells at risk of oxidative stress. Both the decrease of $\Delta\Psi_m$ and increase of ROS are likely to contribute to the dismantling of cells. Other changes after UVB irradiation included changed T-AOC, MDA, and SOD activities. These changes indicated oxidative stress in HLEC mitochondria that caused cell death and gradual formation of cataract.

Besides mitochondrial dysfunction, caspases activities were also triggered after UVB irradiation. Previous studies have shown that released cytochrome from mitochondria could activate caspases to orchestrate the cell death, and in

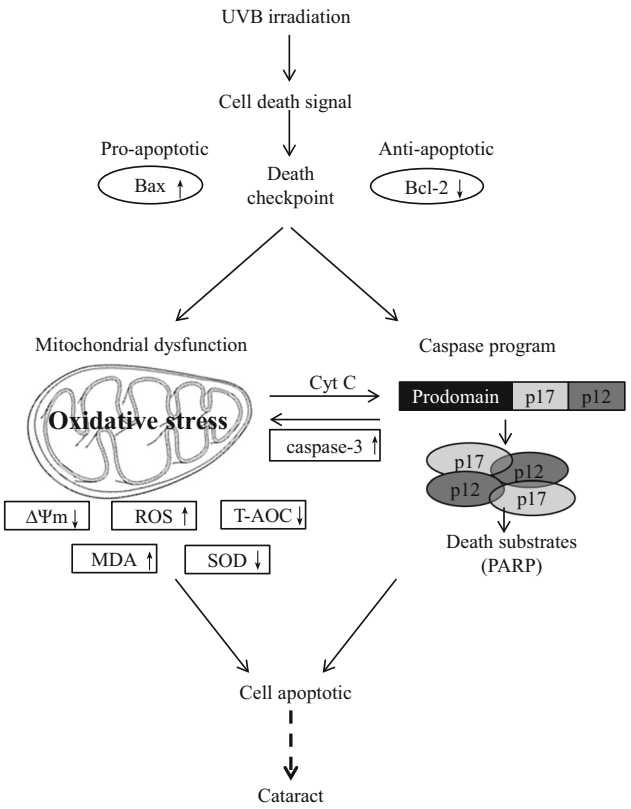

Fig. 6 The hypothesized model of HLEC apoptosis by UVB irradiation. The changes included $\Delta\Psi_m$, ROS, Bax and Bcl-2 were labeled by *black boxes*, whose levels were increased (*up arrow*) or decreased (*down arrow*) after UVB irradiation

turn, activated caspases could also affect the function of mitochondria [12]. Ricci et al. reported that caspase-3 disrupted oxygen consumption induced by complex I and II substrates, but not that induced by electron transfer to complex IV, revealing caspase-mediated loss of mitochondrial function and generation of ROS during apoptosis [13]. Here, we found that caspase-3 activity was increased after UVB irradiation in both time- and dose-dependent manners. This might lead to loss of $\Delta\Psi_m$ and ROS production to induce oxidative stress that caused mitochondrial dysfunction. Increased caspase-3 activity after UVB irradiation might also lead to higher levels of cell death substrates (e.g., PARP). Feldmann Jr. irradiated cultured human lens epithelial cells (HLECs) with UVB light and then determined the effects of the radiation on DNA repair enzyme PARP-1 located in nucleus [35]. The results showed that PARP-1 increased after the cells were treated with UVB light. Hence, we proposed that UVB irradiation activated caspase-3, caused a higher level of PARP-1, and eventually led to HLECs apoptosis.

In summary, we investigated the effects of UVB irradiation on HLECs by examining Bcl-2 and Bax genes, $\Delta\Psi_m$, ROS, T-AOC, MDA, SOD, as well as caspase-3 protein after UVB exposure. After UVB irradiation, the changes in the expression of Bcl-2 and Bax genes may play important roles in regulating HLECs apoptosis. UVB irradiation-initiated HLECs apoptosis may involve complicated mechanisms including mitochondrial dysfunction and caspase-3 activation. The main dysfunction of mitochondria was related to oxidative stress indicated by the decreased $\Delta\Psi_m$ and increased ROS production. These results provided an experimental basis for further investigation of UVB-induced HLECs apoptosis.

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Conflict of interest The authors declare no competing financial interest.

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